10th November 2004

Formalities officer
International preliminary examining authority (IPEA)
European Patent office
D-80298 Munich

Our ref. **144 PCT**
International application No. **PCT/EP03/50891**

Subject : Response to the written opinion of 22 September 2004

Dear Sir,
This letter is in response to the written opinion dated 22nd September 2004.

Claim amendment

Together with this response we send a set of amended claims, which is replacing the previous set (as a new main request).
The claims have been amended in the following way.
Claim 2 has been incorporated in claim 1.
Claim 15 has been incorporated in claim 12.
Since the claims were all present in the application as filed there is an evident basis for the amendments, which have been made. Basically the independent composition and process claims now specify that the antiflocculant and/or antisedimentation agent is xanthan gum.

Correction of cited references

With reference to our earlier response dated September 8th 2004, please note the following corrections with respect to references to the documents D1 to D5.
The international search report listed the following "Y" documents
D1: US 5 866 167
D2: should read as " US 5 387 415" instead of "WO 97/41899"

D3: should read as "JP 07 267876" instead of "US 6 046 178"

D4: should read as "US 6 046 178" instead of "JP 07 267876"

D5: should read as "WO 97/41899" instead of "US 5 387 415"

Hence the arguments submitted previously should be read in light of these corrections. From the arguments summarised below it should be clear to the person skilled in the art that none of the documents D1 to D5 address the problem of flocculation.

Discussion of cited references

D1 describes a process to obtain a non-viable keratinocyte lysate for promoting wound healing. The process consists essentially of growing keratinocyte cells on support, detaching the cells and lysing said cells to obtain a non-viable cell lysate. The present invention describes specifically the problems encountered with such a process, being flocculation and partial sedimentation of the cell lysates (pg. 1, l. 20-23). Cell lysates, resulting from the lysis of the cells, contain many components in various forms, forming an extremely complex mixture of constituents such as proteins, glycoproteins, polysaccharides, lipids, nucleic acids etc. All these components may interact with each other, significantly increasing the possibility for complex formation and flocculation, ultimately resulting in a non-stable solution or suspension and sedimentation of part of these components (pg.2, l.18-23). The present invention provides a solution to this problem by adding agents which prevent the formation of flocculants and stabilize the cell lysate mixture. Such a technical feature is not taught by D1. Thus we submit that the present invention is inventive over D1.

D2 relates to aloe vera juice (active ingredient) containing particles in the form of pellets, which are characterized by dispersing aloe vera juice in a matrix comprising a skeleton formed of a hydrophilic macromolecule. Such amatrix which comprises hydrophilic macromolecules is selected from the group consisting of collagen, gelatin, fractionated gelatin, collagen hydrolysate, gelatin derivatives, plant proteins, plant protein hydrolysates, elastin hydrolysates as well as mixtures thereof. D2 provides this solution to the problem of lumping, often obtained by spray or freeze drying aloe vera powder. In fact this reference specifically indicates (column 2, lines 3-11) that where in order to make conventional products solvating agents are added thereto this

is not desirable in cosmetics. D2 in fact provides a process for maintaining the characteristics of the aloe vera juice without adding all kinds of additional agents. The solution being freezing in some form of matrix. This is completely different from the process and product of the present invention.

In addition, D2 describes how cryopellets increase the shelf life of the active ingredient. D2 also describes the addition of other skeleton forming substances which include xanthan, starch and sugars (pg. 6, l.10-15). These substances are utilized to modify the physical properties of the spray- or freeze-dried matrix.

For a skilled person this teaching fails to provide a proper motivation nor suggest a proper solution to the problem of present invention which being stabilizing the cell lysate composition. Thus the present invention is inventive over D2.

D3 relates to a technique for stabilization of a cell growth factor. The platelet derived growth factor (PDGF) are polypeptides whose stability or protein conformation is maintained by addition of polysaccharides thus protecting the activity of the protein. The biological stability of these factors is increased by adding water-soluble polysaccharide such as alginate or xanthan gum OR water soluble vinyl polymer such as carboxyvinyl polymer. However, D3 does not suggest the use of polysaccharides to increase the physical stability (flocculation, sedimentation) of such growth factors.

D3 relates to the use of certain agents to stabilise the biological activity of an isolated protein and therefor does not disclose any technical feature, which provides a solution to the problem of present invention i.e. physical stabilisation of a complex mixture of molecules. Thus we submit the present invention is inventive over D3.

D4 relates to a method for treating wounds with starch hydrolysate composition including trace elements to beneficiate the wound healing processes. The composition, apart from above the mentioned constituents, contains sterile water, added to form an emulsion of desired viscosity (pg. 2, l. 55-57). Starch hydrolysate represents a mixture of carbohydrates, mostly preferred are maltodextrins, and are used as film forming agents. The advantage of starch hydrolysate apart from forming films is that they possess antimicrobial effects (pg. 4, l. 20-36). This document does not describe the use or the production of cell lysates to be used in wound healing.

This document is far fetched in terms of the offering a solution to the technical problem stated in the present invention. Thus we submit the present invention is inventive over D4.

D5 relates to a medicament containing a biopolymer matrix comprising gelatin cross linked with oxidised polysaccharides. The material is also used for controlled release of drugs. Oxidised form of xanthan is used for forming the gel-matrix. Similar to D4, the D5 teachings are not related not even remotely to the present invention and thus provides no motivation for a skilled person to utilise the concepts of D5 for deriving the technical aspects of the present invention.
Thus we submit the present invention is inventive over D5.

Additional references

In addition to above arguments (partly submitted in our previous response), we here present the following further arguments.
Whereas at first sight it may indeed seem obvious to use an antiflocculant/antisedimentation agent to prevent flocculation or sedimentation, the choice which agent performs the desired function is by no means obvious. Cell lysates are highly complex mixtures of proteins, lipids polysaccharides and many other compounds, often in a particulate form. The interactions between these compounds, and how these interactions lead to flocculation and subsequent sedimentation are very hard to predict. Likewise, the way in which potential antiflocculants interact with the compounds of the lysate are also hard to predict and it is clear that prevention of these undesirable physical processes can not be achieved by just adding a viscosity-increasing agent. In our research leading to the present invention, we have found that many of the "obvious" antiflocculant/antisedimentation agents failed to prevent flocculation/sedimentation.
By serendipity we have also tested xanthan gum. To the man skilled in the art, it would however be highly surprising if xanthan gum would inhibit flocculation, since it is well known that xanthan gum actually induces flocculation in many pharmaceutical and food formulations. In order to substantiate this point the Examiners' attention is drawn to the abstracts which we have annexed to this letter. The 9 abstracts of references from scientific journals all indicate that xanthan gum is used for its flocculation properties both in food and pharmaceutical applications.

The fact that xanthan gum, alone or in combination with maltodextrin, inhibited flocculation and sedimentation in the cell lysates of the present invention is therefore to be considered a surprising and unexpected finding, and using this agent for that purpose is certainly not obvious.

Furthermore, the fact that xanthan gum might display these desirable properties is not thought nor suggested in any of the references cited by the examiner. D1 describes the process to obtain keratinocytes lysates and the flocculation problems encountered in such a process, it does however not teach how to solve these problems. D2 indeed describes the use of xanthan gum. However, it does not suggest xanthan gum has useful antiflocculant properties, since the function described for xanthan gum in D2 only relates to its properties as a matrix former, enhancing the solubilization of dried Aloë vera juice. In fact, D2 does not even touch the problem of flocculation in the juice before drying, which apparently doesn't seem to be a problem to be overcome in that process. In neither D3, nor D4 nor D5, the problem of flocculation within cell lysates is discussed, nor is the possible use of xanthan gum for preventing flocculation suggested.

Conclusion

IN view of the above we submit that the present invention is inventive over documents D1 to D5.

Sincerely yours

Cornelis Schüller
(Authorized Representative)

Annex : - Set of amended claims
- 9 abstracts

Claims (amended claims, 10 November 2004)

1. A pharmaceutical composition comprising a non-viable cell lysate and at least one antiflocculant and/or antisedimentation agent(s), wherein the antiflocculant and/or antisedimentation agent is xanthan gum.
2. The pharmaceutical composition of claim 1, wherein the antiflocculant and /or antisedimentation agent is a combination of xanthan gum and maltodextrin.
3. The pharmaceutical composition of claim 1 or 2 further comprising a buffering agent.
4. The pharmaceutical composition of claims 1 to 3 in a dried form.
5. The pharmaceutical composition of claims 1 to 3 in a freeze-dried form.
6. The pharmaceutical composition of claims 1 to 5, wherein the cell lysate is a keratinocyte cell lysate.
7. The pharmaceutical composition of claims 1 to 6, wherein said composition further comprises a pharmaceutically acceptable carrier/excipient/vehicle.
8. The pharmaceutical composition of claims 1 to 7 for the purpose of promoting wound healing, wherein said composition comprises a non-viable cell lysate and a pharmaceutically acceptable vehicle.
9. The pharmaceutical composition of claims 7 or 8, wherein said pharmaceutically acceptable vehicle is a dry powder, a suspension or a solution.
10. The pharmaceutical composition of claims 7 or 8 wherein said pharmaceutically acceptable vehicle is a gel, cream, ointment or a biocompatible matrix.
11. A process for the production of a homogenized pharmaceutical composition comprising the steps of growing, lysing and drying the cells, which process is characterized in that, immediately after lysing, antiflocculant and/or antisedimentation agents are added to stabilize the cell lysate mixture, wherein the antiflocculant/antisedimentation agent is xanthan gum.
12. A process of claim 11 wherein drying the cells is by freeze-drying.
13. A process of claims 11 or 12, wherein cell the lysate is a keratinocyte cell lysate.
14. A process of claims 11 to 13 wherein the antiflocculant and/or antisendimentation agent is a combination of xanthan gum and maltodextrin.
15. Use of the composition of claim 1 to 10 for the treatment of burn wounds or skin ulcers.